Examination of monodispersed artificial particles similar to *C. parvum* oocysts in size as the removal surrogate of *Cryptosporidium*

Han-Seung Kim, Junji Shikiya, Michihiro Akiba and Shoichi Kunikane

ABSTRACT

This study investigated the feasibility of using artificial particles, MX-500 and SX-500, similar to *Cryptosporidium parvum* oocysts in size, as surrogate tracers of *Cryptosporidium* removal in water treatment. For this purpose, coagulation-filtration experiments were conducted for the two kinds of artificial particles and *C. parvum* oocysts under such conditions as coagulant (polyaluminium chloride) concentration of 8 mg l^{-1}, influent of 1,000 counts ml^{-1} and kaolin turbidity of 10 units. The results showed that the turbidity removals were almost same for the three cases at 95%, while the particle removals differed: 1.92, 1.74 and 2.00 log_{10} units for MX-500, SX-500 and *Cryptosporidium* oocysts, respectively. The un-coagulated particle counts in the coagulation chamber were 300 counts ml^{-1} for SX-500 and 240 counts ml^{-1} for MX-500 and *C. parvum* oocysts, which indicated that the coagulation efficiency of the three kinds of particle was similar. In the examination of the filtrate counts, similar filtration behaviour was found between both artificial particles and *C. parvum* oocysts over the filtration running time with correlation coefficients (R^2) over 85%. These similarities in the coagulation and filtration characteristics of the artificial particles and *C. parvum* oocysts suggest that the particles of similar size to *C. parvum* would be reliable removal surrogates of *C. parvum* oocysts for the evaluation of *C. parvum* oocyst removal in water treatment processes.

Key words | artificial particle, coagulation, *Cryptosporidium*, filtration, surrogate, water treatment

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INTRODUCTION

Outbreaks of cryptosporidiosis have been increasingly reported in recent years; the main reasons for the incidences were considered to be *Cryptosporidium* contamination upstream of water sources and suboptimal operation of water treatment plants (Hayes *et al.* 1989; MacKenzie, *et al.* 1994). Moreover, public water supply was thought to be a vehicle of transmission responsible for the epidemic of cryptosporidiosis (Duncanson *et al.* 2000). With an improvement in detection technology, occurrences of *Cryptosporidium* in water sources have been reported throughout the world (Roach *et al.* 1993; Hsu *et al.* 1999; Robertson *et al.* 2000; Hashimoto *et al.* 2001). This implies that *Cryptosporidium* oocysts exist in almost all surface water sources. In order to control the contamination of drinking water with *Cryptosporidium*, therefore, routine surveillance and monitoring of *Cryptosporidium* levels in both raw and treated waters is required and optimum conditions in water treatment processes should also be maintained.

However, it usually takes considerable time and cost to determine and measure the level of *Cryptosporidium* oocysts routinely. And no simple method utilizing common skills has been developed so far. Moreover, the direct use of *Cryptosporidium* in evaluating the removal performance of water treatment processes is not desirable because of a possible risk of infection with *Cryptosporidium*. Using reliable surrogates is an alternative way of monitoring the level of *Cryptosporidium* and checking the removal performance of the treatment processes (Li *et al.* 1997). Turbidity has been regarded and

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used as a convenient index of *Cryptosporidium* removal for its ease and rapidity of measurement. But it is controversial whether turbidity is reliable as a surrogate index of *Cryptosporidium* removal, because both poor correlation and good correlation have been observed between turbidity and *Cryptosporidium* (LeChevallier & Norton 1992; Hsu & Yeh 2003). Some kinds of algae have also been suggested as a removal index for *Cryptosporidium* in water treatment processes for their wide occurrence in surface water sources and similar removal rates to *Cryptosporidium* (Akiba *et al.* 2002).

In this study, two kinds of monodispersed artificial particles, MX-500 and SX-500, which are similar in size to *Cryptosporidium parvum* oocysts and have a sphere shape with a diameter of around 5 μm, were examined as surrogate tracers of *Cryptosporidium* removal through a series of experiments. The objective of this study was to investigate the removal behaviour of the artificial particles and to evaluate the possibility of using them as potential surrogates of *Cryptosporidium* removal in water treatment. The experiments were conducted through jar tests and bench-scale coagulation-filtration tests. The coagulation and filtration efficiencies of the artificial particles and *Cryptosporidium* were examined and compared to evaluate the similarity between those particles.

MATERIALS AND METHODS

C. parvum oocysts and the artificial particles

Cryptosporidium parvum oocysts used in this study were commercial ones obtained from Kanto-Chemical, manufactured by Tech-Lab (USA). The oocysts were contained in a 5 ml vial in an inactivated state with formalin-fixed and phosphate-buffered. Density of the oocysts was known to be between 1.05 and 1.10 g cm^{-3} and diameter was between 4 and 6 μm. The nominal concentration of the commercial *Cryptosporidium parvum* oocysts was 2×10^7 oocysts.

MX-500 and SX-500, the monodispersed particles, were obtained from Sohken-Chemical. MX-500 is a polymethyl-*meta*-acryl sphere, which has a density of 1.19 g cm^{-3} and a diameter of 4.9 μm. SX-500 is a polystyrene sphere, which has a density of 1.05 g cm^{-3} and a diameter of 5.0 μm. Prior to introducing the oocysts and the artificial particles into the experiments, the initial concentrations were counted using hemocytometer counts with a microscope (BX-60, Olympus) at a magnification of 200 times.

Jar tests

Test waters were prepared for kaolin, MX-500 and SX-500 each with a turbidity of 10 units using distilled water. Each test water of 500 ml was then decanted into a beaker, followed by pH-adjustment. Jar tests were carried out using a jar tester (T-6 S, Sugiyamagen) at a pH range from 3 to 10 according to the procedure of rapid mixing at 100 rpm for 5 min, slow mixing at 40 rpm for 25 min and sedimentation for 30 min. In order to find an optimum addition concentration of coagulant (polyaluminium chloride), the jar tests were carried out at ALT ratio (ratio of aluminium dosage to turbidity) from 0 to 0.1. The test waters contained MX-500, SX-500 and *C. parvum* oocysts at 1,000 counts ml^{-1} with 10 mg l^{-1} kaolin (10 units) and alkalinity of 40 mg l^{-1} as $CaCO_3$. Zeta potential was measured with a zeta potentiometer (ZEECOM, Microtech Nition) after rapid mixing. Turbidity of the supernatant was measured after sedimentation with a turbidimeter (SET-PT-706D, Mitsubishi Chemical).

Coagulation-filtration tests

The tests were conducted for MX-500, SX-500 and *C. parvum* oocysts individually, under the operation conditions shown in Table 1 with the experimental setup shown in Figure 1. Raw waters were made for each kind of particle. Kaolin was added to the raw waters in order to make the initial turbidity about 10 units. The alkalinity of the raw water was made up to 40 mg l^{-1} as $CaCO_3$ using a $NaHCO_3$ stock solution. 0.1 N of NaOH and HCl solutions were used for pH adjustment of raw water so that the pH was set to around 7.0. Initial concentrations of the particles in the raw water were 1,000 counts ml^{-1}.

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Table 1 | Operation conditions

Coagulation	Mixing speed (rpm)	100
	Chamber volume (l)	1.5
	Retention time (min)	8.3
	PAC concentration (ppm)	8
Filtration	Column diameter (mm)	30
	Filter depth (mm)	100
	Flow rate (ml min^{-1})	180
Filter media (sand)	Diameter (mm)	0.6
	Uniformity	1.4
	Void rate of filter	0.38

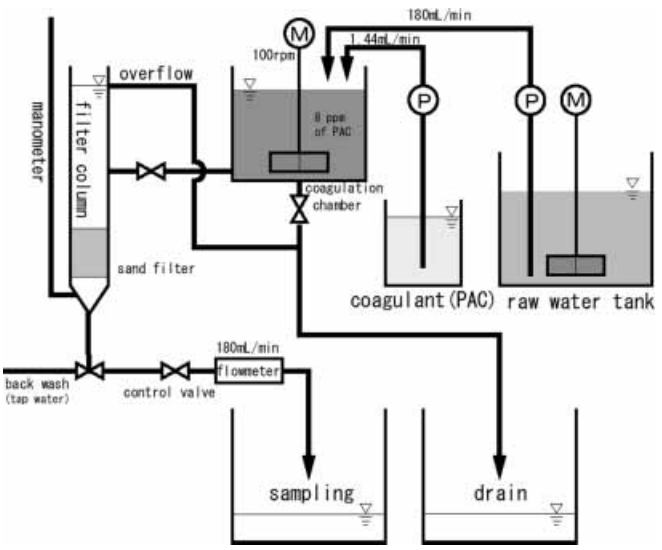

Figure 1 | Schematic of the experimental setup.

Diluted to 1,000 ppm with distilled water, PAC (poly-aluminium chloride, 10% as Al_2O_3) as a coagulant was fed into the coagulation chamber (1.5 l) at a flowrate of 1.44 ml min^{-1} using a peristaltic pump. The raw water was also fed in to the coagulation chamber at a steady flowrate of 180 ml min^{-1} using a master flex pump.

After the raw water was mixed with the coagulant, the mixture was introduced to a filtration column. The filtration flowrate was maintained at 180 ml min^{-1} (or 360 m day^{-1}) by controlling an outlet valve of the filtration column. During the operation, the un-coagulated particle counts in the coagulation chamber were measured in order to investigate the coagulation efficiency for each kind of particle. Filtrate samples were taken at scheduled time intervals (every 1 min from 0 to 5 min, then every 10 min till 180 min) from the outlet of the filtration column. Turbidity of the filtrates was measured as soon as the samples were taken. As for the particle counts, 10 ml of the samples were taken to a 15 ml centrifugal tube to centrifuge at 1,500 × g for 10 min. By drawing out 9 ml of supernatant, the samples were concentrated by 10 times, and the counts were estimated measuring 0.05 ml of the concentrated samples with a microscopic examination at a magnification of 200 and 400 times.

Head loss was read from the manometer installed alongside the filtration column at each sampling time during the filtration tests. A total of 65 cm could be measured as effective head loss corresponding to the water depth from the surface of the water in the filtration column to the top of the sand filter layer. The head loss was calculated from the difference between the water levels in the filtration column and the manometer.

After each filtration test finished, the sand filter layer was taken out of the filtration column and divided into five portions by depth. Each portion was taken into a beaker, and then 500 ml of distilled water was added to each beaker. The mixtures were stirred thoroughly for 10 min using a magnetic stirrer to separate the attached matter from the sand. The particle counts of each suspension were measured without any concentration processes. Suspended solids were measured by filtering 300 ml of the suspension with a GF/B filter, drying at 105°C for 2 h and weighing.

RESULTS AND DISCUSSION

Zeta potential changes with aluminium dosage

The zeta potentials of MX-500, SX-500 and kaolin over a pH range from 3 to 10 are displayed in Figure 2. As the pH

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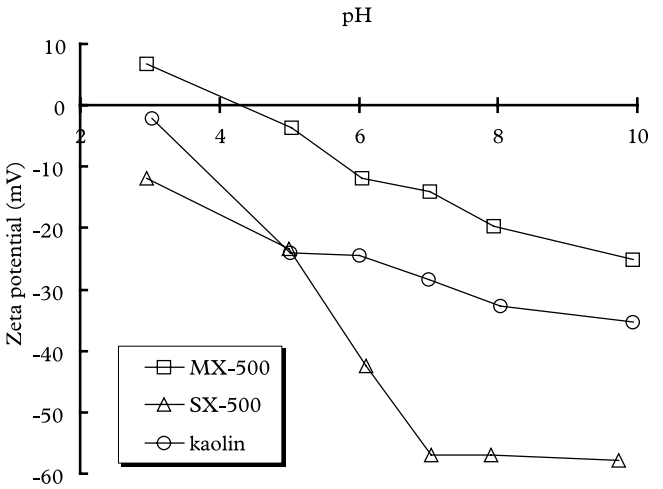

Figure 2 | Zeta potential of MX-500, SX-500 and kaolin in the pH range 3–10.

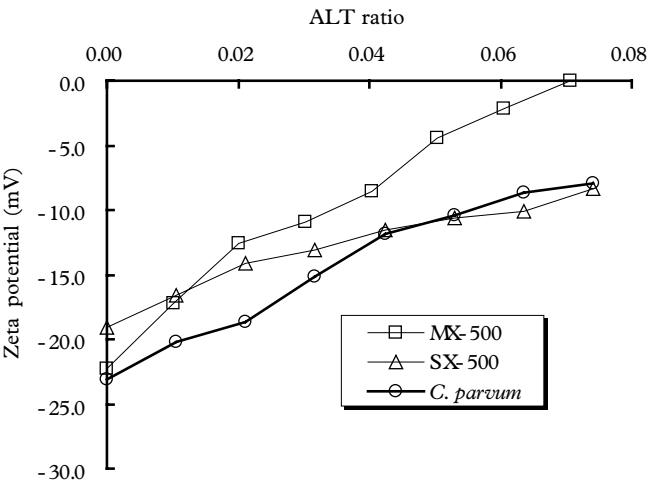

Figure 3 | Variation of zeta potential with the change in ALT ratio at pH 7.

increased, the zeta potentials appeared to decrease for all particles. The zeta potentials at pH 7 were −14.1, −56.9 and −28.4 mV for MX-500, SX-500 and kaolin, respectively. For *C. parvum* oocysts, a zeta potential ranging from −20 to −30 mV has been reported at neutral pH (Drozd & Schwartzbrod 1996). The differences in zeta potential between the particles possibly stem from the surface characteristics of each particle due to its surface functional group. The coagulation process generally depends on the surface properties of the particles involved (Tambo & Watanabe 1979), and a favourable zeta potential has been known to be between −10 and 10 mV for effective coagulation (Tambo 1964). This indicates that the particles considered here exist in a stable state at neutral pH and no coagulation process occurs. In other words, addition of coagulants is needed for coagulation.

Optimum ALT ratio for the filtration tests

Variations of the zeta potentials of MX-500, SX-500 and *Cryptosporidium* oocysts at an ALT ratio between 0 and 0.08 are described in Figure 3. Although the zeta potentials increased with the increase in ALT ratio, the differences in the zeta potentials between the particles did not appear to be so significant, as described in Figure 3. It is probably because kaolin was such a dominant component in the test waters, compared to MX-500, SX-500 and *Cryptosporidium* oocysts, that the characteristics of each particle seemed to be hidden by the kaolin particles. The zeta potentials of −8.6 mV for MX-500, −11.6 mV for SX-500 and −11.9 mV for *Cryptosporidium* oocysts were observed at an ALT ratio of 0.04 corresponding to 8 mg l^{-1} of PAC. Although the zeta potential increased with the increase of ALT ratio, which leads to a better coagulation state, a zeta potential of around −10 mV is known to be appropriate for the formation of micro-flocs that are more favourable than macro-flocs in the direct filtration process (Tambo & Ogasawara 1985). From this result, a coagulant dosage of 8 mg l^{-1} was applied to the filtration tests.

Investigation of un-coagulated particle counts in the coagulation chamber

The un-coagulated particle counts were measured in the coagulation chamber, in order to evaluate the coagulation efficiencies for the particles. Counting was conducted at the operation time of 30 min which was considered at a steady coagulation state. The un-coagulated particles here indicate the particles that exist not in a coagulated form but in a singular form, which means the coagulation

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Table 2 | Un-coagulated particle counts in the coagulation chamber

	Particle counts (counts ml^{-1})
MX-500	300
SX-500	240
C. parvum oocysts	240

efficiencies of the particles. The results in Table 2 show that 30% for SX-500 and 24% for MX-500 and *C. parvum* oocysts turned out to exist in a singular state. Consequently, 70 to 76% of the particles were introduced into the filtration column after the coagulation process. Although a slightly lower coagulation efficiency was shown in SX-500 compared with the other particles, the difference was not significant and the coagulation efficiencies of the three kinds of particle could be considered to be of the same degree.

Filtration and removal behaviour of particle counts and turbidity

The particle counts and turbidity of the filtrates for MX-500, SX-500 and *C. parvum* are displayed in Figures 4 and 5, respectively. A filter ripening period is shown for the first 5 min from the start of the filtration running, and then a stable filtration period lasting about 40 min. After breakthrough began to appear at a running time of 45 min, particle counts and turbidity in the effluents gradually increased with the filtration time. In Figure 4, the artificial particles showed almost the same filtrate counts as *C. parvum* at the filtration time of 100 min. This implied that the artificial particles and *C. parvum* behaved in almost the same way in the filtration process.

The removal rates of MX-500, SX-500 and *C. parvum* with filtration time were calculated from the filtrate counts and the influent counts. A removal rate of around 2 $\log_{10}$ unit was observed during the stable filtration time of 40 min for the two artificial particles and *C. parvum*. This implied that the sand filter examined in this experiment

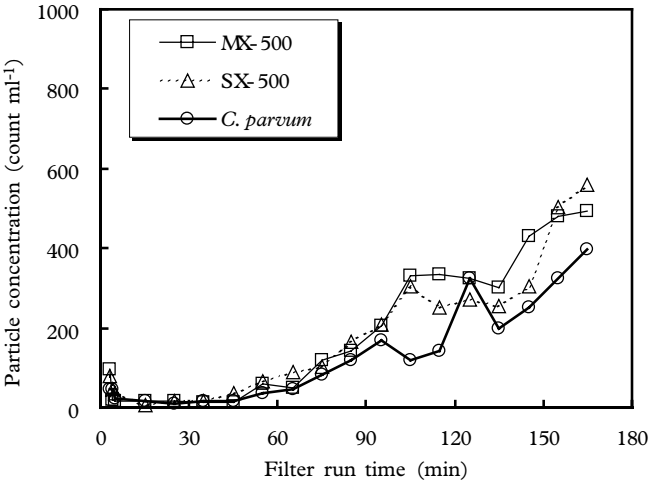

Figure 4 | Particle concentration of the filtrate during a filter run.

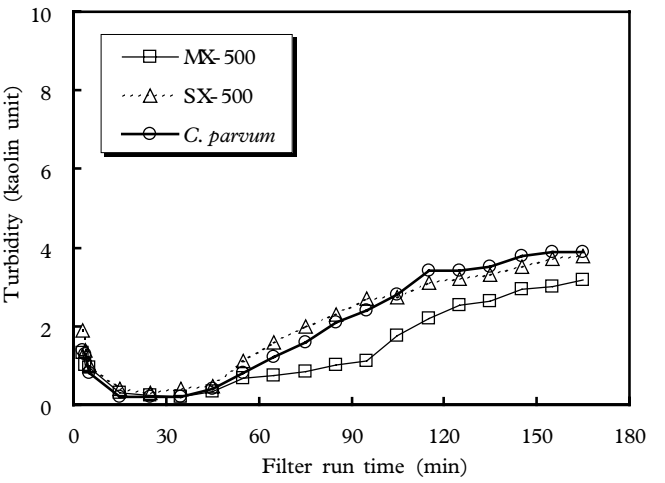

Figure 5 | Turbidity profile of the filtrate during a filter run.

had a treatment capacity of 1 $m^3\ m^{-2}$ of the influents per 1 cm of the sand filter layer: 40 min of stable filtration multiplied by 15 m h^{-1} filtration flowrate gives 10 m (or 10 $m^3\ m^{-2}$) specific volume treated through 10 cm (or 71 cm^3) of sand filter with a removal rate of around 2 $\log_{10}$ units. Then, the removal rates decreased to 1 $\log_{10}$ unit after an operation time of 80 min and to 0.5 log unit after 120 min; a halving of removal rates every 40 min.

Although the turbidity profiles of each particle showed a smooth increase with time during the breakthrough period, fluctuations were observed in the particle

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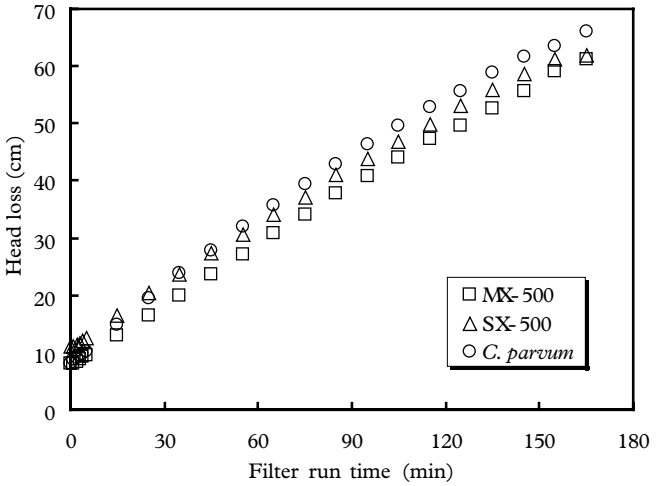

Figure 6 | Variation of pressure drop (head loss) during a filter run.

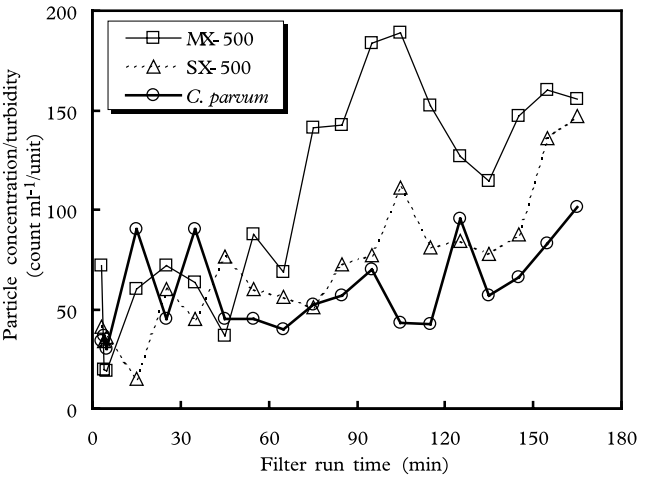

Figure 7 | Variation of particle concentration to turbidity during a filter run.

count profiles after 100 min of filtration. One of the possible reasons is thought to be an irregular breakthrough of agglomerations of the coagulated flocs that were formed in the filter layer. As the deposition of the flocs in the filter layer progressed, the spatial volume of the filter was reduced, resulting in the increase of the head loss (or pressure drop) as shown in Figure 6. The head loss can be converted to pressure drop (head loss of 10 cm corresponds to 97.9 Pa at 20°C) and the resistance can be obtained from the relationship $R = \Delta P/\mu J$, where R is the resistance, ΔP the pressure drop, μ viscosity and J flux. The fluctuation occurred at a head loss of 40 to 50 cm, which corresponds to a resistance of $9.20–11.50 \times 10^6$ m^{-1}. As the filtration progressed, the filtration resistance increased linearly to reach a certain level (10^7 m^{-1}) which seemed to be a critical resistance. And when the resistance exceeded the critical level, the increased spatial velocity and shear force apparently caused the agglomeration of the flocs trapped in the filter media to come off into the filtrate irregularly. In this case, the agglomerated flocs might include lots of individual particles that could not be fully reflected in the filtrate turbidity, resulting in the same turbidity but different particle counts in the filtrate. These results imply that filtration behaviour can be observed more precisely by measuring the particle counts than by checking turbidity in the filtrates.

The variation in the ratio of particle counts to turbidity with filtration time is illustrated in Figure 7, which explains the behaviour of the rate of particles in turbidity of one unit. It was observed that the ratio increased with the filtration time. This meant that the filtrates included more particles at the same turbidity as the filtration progressed. This inclination appeared clearly during the breakthrough period. This finding suggests that both turbidity and particle counts should be examined for the better evaluation of the efficiency of *Cryptosporidium* removal in the filtration processes.

Comparison of the filtrate counts between MX-500, SX-500 and *C. parvum*

The particle counts of the filtrates for *C. parvum* and the artificial particles are described in Figure 8. The data points were made by plotting the particle counts for MX-500 and SX-500 on the *y*-axis and for *C. parvum* on the *x*-axis, which were the particle counts of the filtrates at the same given sampling times. The ratios of the filtrate counts of MX-500 and SX-500 to those of *C. parvum* were 1.33 and 1.27 with correlations (R^2) of 86% and 88%, respectively. The filtrate counts of the artificial particles appeared to be slightly higher than those of *C. parvum* oocysts. This implied that the artificial particles had a tendency to be a little easier to leak from the filter layer

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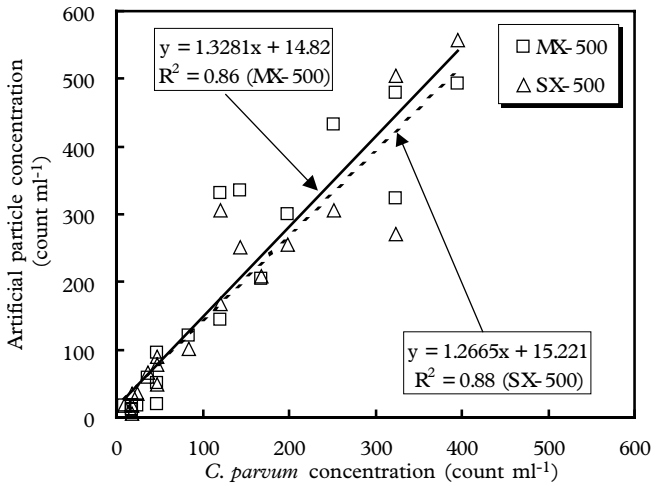

Figure 8 | Correlation between the concentrations of the artificial particles and *C. parvum*.

than the *C. parvum* oocysts. In the figure, the data points appeared relatively nearer to the approximation line in the lower count region than in the higher. Considering that lower particle counts were observed during the stable filtration period, it is expected that similar filtration behaviour between the artificial particles and *C. parvum* oocysts would be observed during normal filtration operation in real water treatment plants. These similarities in filtration behaviour and slightly higher counts in the filtrates suggest that the two artificial particles could be regarded as reliable surrogates of *C. parvum* removal.

Deposition of each kind of particle in the filter layer

Table 3 summarizes the particle counts and suspended solids of MX-500, SX-500 and *C. parvum* deposited in the sand filter during the filtration running. The higher deposition rates appeared in the upper layer of the filter, while the deposition rate was gradually reduced along the filter depth. This may be due to the stratification of the filter media whereby the coarse media is located lower down and fine media higher up, and the reduction of zeta potential in the filter with depth that results in a decrease in the attachment strength between the filter media and the coagulation flocs (Ebie & Doi 1998; Logan *et al.* 2001).

Compared with the total particle counts of *C. parvum*, those of MX-500 and SX-500 were 92.1% and 97.5%, respectively. A slightly lower deposition rate was observed in MX-500 than in the other particles. On the other hand, no significant differences were observed in the deposited suspended solids between the three kinds of particles; 98.9% for MX-500 and 103% for SX-500, compared with *C. parvum*. It is probably because the dominant component of turbidity in the raw water was kaolin, and therefore the differences between each particle did not

Table 3 | Particle counts ($\times 10^6$ counts) and suspended solids (mg) deposited in the sand filter layer during a filter run

	MX-500		SX-500		*C. parvum*	
Depth (cm)	Particle counts	Suspended solids	Particle counts	Suspended solids	Particle counts	Suspended solids
0~2	1.65	82.9	1.66	86.3	1.80	92.8
2~4	1.17	58.6	1.35	62.8	1.58	56.6
4~6	1.20	52.9	1.32	52.8	1.20	44.1
6~8	0.72	42.7	1.05	40.2	0.90	39.1
8~10	0.72	25.4	0.40	32.2	0.45	32.9
Total	5.46	262.5	5.78	274.3	5.93	265.5

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appear in the amounts of suspended solids deposited. The observation from the particle counts and the suspended solids deposited at each depth of the filter layer revealed that the deposition behaviour of the three kinds of particles seemed to be alike along the filter depth.

CONCLUSIONS

This study investigated and evaluated the feasibility of using the monodispersed artificial particles, MX-500 and SX-500, with a diameter of around 5 μm similar to *C. parvum* oocysts, as surrogate tracers of *Cryptosporidium* removal in water treatment by conducting coagulation and filtration experiments. Although the physical characteristics of the two particles were slightly different from *C. parvum* oocysts, they demonstrated similar coagulation and filtration behaviour to *C. parvum* oocysts. The results obtained from this study are summarized as follows:

1. The filtrate counts of the artificial particles and *C. parvum* oocysts showed almost the same pattern throughout the operation period.
2. The removal rate of the particles reached around 2 $\log_{10}$ units during the stable filtration period.
3. The un-coagulated counts in the coagulation chamber and the deposited counts in the filter layer showed little differences between the three kinds of particles, which implies that the efficiency of coagulation and filtration for the artificial particles was of the same degree as the *C. parvum* oocysts.
4. Removal behaviour of *C. parvum* oocysts was well described by monitoring the artificial particle counts in the experiments, which suggests that those particles of a similar size to *C. parvum* oocysts are reliable indices.

These results show that the similarity in particle size is considered to be an important parameter in evaluating the surrogates of *C. parvum* oocyst removal in water treatment plants. Therefore, the use of the proposed particles is believed to be a reasonable way of evaluating the efficiency of treatment facilities for the removal of *C. parvum* oocysts.

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